THE EFFECTS OF THE PROTOZOAN FAUNA OF TRICKLING FILTER BALLAST UPON SALMONELLA SPECIES

by

WILFRED V. G. CHONG

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INTRODUCTION

The role of the trickling filter in a sewage treatment plant has received much attention since the turn of the century when the Lawrence Experiment Station (1885 to 1900) started to experiment on early sewage filters. The problem of sewage disposal evolved from the rise of large cities. Many outbreaks of typhoid fever in the past were traced to polluted water supplies. When one considers the carrier status of many individuals who have had typhoid fever, it is understandable why it is important to dispose of sewage properly and safely.

Investigators in the past had observed the fauna of the biologic film on the stones of trickling filter ballast and had shown that trickling filters were effective in removing large percentages of bacterial cells in sewage. Other investigators had proven the bactericidal effects of flagellates and ciliates. However, no work could be found on the destruction of Salmonella species by protozoa.

The purpose of this research was mainly an attempt to determine if one of the more predominant protozoans present in the biologic slime is effective in destroying Salmonella species, using the coliform bacteria as control organisms for comparison.

REVIEW OF LITERATURE

The Lawrence (Massachusetts) Experiment Station, during 1885 to 1900, did considerable work on early sewage filters. This

work has been summarized by Phelps (1944). These filters were of the intermittent sand type which removed 98 to 99 per cent of the organic matter and bacteria. Phelps (1944) stated, "At the Lawrence Experiment Station, it was our regular custom to invite visitors, sometimes distinguished visitors from abroad, to drink one of these effluents served from a cut-glass pitcher, in cut-glass tumblers." The filter must have been very efficient to prompt the Lawrence Experiment Station officials to do what they had done. Phelps described another early experiment performed at the Lawrence Experiment Station in which the passage of Eberthella typhi (Salmonella typhosa)[1] through sand filters was compared with that of Bacterium coli (Escherichia coli). Even with an excessively high concentration of E. typhi (S. typhosa) in the influent, it was almost impossible to force viable typhoid organisms through the filter. However, Bacterium coli (Escherichia coli) did appear at times in quantities of 0.1 per cent of the applied concentration.

Clark (1907) in his experiments found that sprinkling filters were not a substitute for sand filters, which removed practically all the organic matter suspended in sewage. He theorized that sprinkling filters were simple devices for the quick oxidation of all putrefying matter in sewage while allowing the larger body of the stable or slowly decomposing materials to pass along

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[1] The names of the organisms mentioned in this thesis are those used by the various authors cited in the literature. The designation according to Bergey's Manual of Determinative Bacteriology, 6th Edition, Baltimore: Williams and Wilkins, 1948, follows in parentheses each obsolete name.

with the effluent.

About the same time, Jordan et al. (1904) in their classic study of the longevity of E. typhi (S. typhosa) in streams found that the organisms remained viable after four to eight days when introduced into Lake Michigan water. They found the survival time to be generally lower when the concentration of contaminants was high. In sterilized tap water, the longevity had increased to 25 days, demonstrating the effect of competitive organisms against the typhoid bacillus.

Russell and Fuller (1906) discovered that E. typhi (S. typhosa) survived from eight to ten days in pure surface water, but only three to five days in sewage. When inoculated surface water in collodion sacs was immersed in flowing sewage, survival time was 10 to 14 days. When organisms were suspended in sewage and immersed in flowing surface water, viable organisms were recovered for three to five days.

While working with water, Houston (1913) found that cultivated typhoid bacilli persisted for five to nine weeks; whereas, organisms added directly from a typhoid patient survived for only one to three weeks. He also discovered that survival time varied from nine weeks at zero degrees centigrade to two weeks at 37° C.

Ballentyne (1930) listed low temperature, a minimum of nutrient material, a minimum or absence of sodium chloride, and a very high bacterial concentration as factors which allowed survival of bacteria, but were unfavorable for bacterial growth.

It was shown by Whipple and Mayer (1906) that the lack of

oxygen was harmful to E. typhi (S. typhosa) suspended in sterile water. However, growth occurred when nutrients were added. On the contrary, Heukelekian and Schulhoff (1935) found that aeration was detrimental to E. typhi (S. typhosa) in nonsterile domestic sewage.

Lackey (1925a) in studying the fauna of Imhoff tanks observed the presence of all three of the saprophytic classes of protozoans. Flagellates comprised the greatest number of genera, the Rhizopoda the fewest. He found the most common forms present to be either facultative or obligate anaerobes.

In his studies on the protozoan fauna of sprinkling filter beds, Lackey (1925b) observed that there was no great difference between the number of organisms (total animal population) in old and new zoogleal films on the filter ballast. It was found that nematodes may be grown in the laboratory on modified nutrient agar. Rudolfs (1931) also observed the common protozoans in filter beds. He found Opercularia sp. to be the most numerous organism present.

By means of staining technics, Huntemuller (1905) observed the ingestion of bacteria by certain flagellated protozoans. Fehrs (1906) demonstrated that typhoid bacilli could live seven days in unsterilized tap water, 46 days in the same water sterilized, and 13 days in the water inoculated with flagellated protozoans after sterilization. Stokvis (1909) also observed that flagellates contributed actively in the extermination of bacteria in water.

In a later experiment, Stokvis and Swellengreble (1911)

proved that infusorians had the same bactericidal power as flagellates. Suspensions containing Bacillus megatherium, Vibrio comma, the Dunbar and El Tor vibrio (thought to be a variety of Vibrio comma), E. typhi (S. typhosa), and Spirillum sp. disappeared when Colpoda cucullus Muller was added. They discovered that the bactericidal effect was not due to the production of toxic substances. Sunlight did not stop the antagonistic action. However, temperatures below 10° C. and above 30° C. and the absence of oxygen proved to be unfavorable to C. cucullus.

Butterfield and Purdy (1931) in a series of experiments showed the ecological relationship between the bacterial and protozoan populations. Bacteria inoculated into sterile sewage multiplied greatly. When a pure culture of Colpidium sp. was added, the bacterial count decreased rapidly. When the protozoan population was sufficiently reduced, a sudden increase of bacteria resulted. The same results were obtained when Paramecium sp. was added instead of Colpidium sp. In the same experiment a bacteria-free culture of Paramecium sp. was obtained. However, the protozoan died before the fourth day, proving that the organism was not capable of living in sterile sewage.

It was discovered by Rudolfs, Chamberlain, and Heukelekian (1931) that most of the protozoans feeding on bacteria were found in the upper parts of the trickling filter bed. The upper portion of the filter removed up to 90 per cent of the B. coli (E. coli) originally present.

In their studies on the survival of Bacterium typhosus (S. typhosa) in surface waters and sewage, Heukelekian and Schulhoff

(1935) observed that the initial increase in the number of B. typhosus (S. typhosa) in sewage was due to favorable temperature, the presence of a food supply, and a high ratio of the bacteria to contaminants.

EXPERIMENTAL MATERIALS

Source of Materials

The source of all sewage, effluents, and ballast stones was the sewage treatment plant of the Fort Riley Military Reservation, Kansas. This plant consists of a primary sedimentation tank, two trickling filters (one of which is not in operation), a secondary sedimentation tank, a digestion tank, and drying beds. Studies were limited to the trickling filter.

Measurements of the filter were found to be as follows:

Depth at periphery..... 48"
Depth at center..... 52"
Diameter of filter..... 82'

The average winter temperatures of the sewage were found to be as follows:[1]

Raw sewage influent..... 16° C.
Trickling filter effluent..... 12° C.
Final plant effluent..... 13° C.

The ballast is composed of stones of St. Joseph, Missouri, granite. The average size of the stones is approximately one and

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[1] Summer temperatures are approximately 5.5° C. warmer in each case.

one-half to two inches in diameter.

Experimental Trickling Filter

A series of five trickling filters was constructed in the laboratory as illustrated in Plate I. Dimensions of the experimental filters were as follows:

Depth of filter..... 10"
Diameter of filter..... $3\frac{3}{4}$"

Methyl Cellulose

Ten per cent methyl cellulose was obtained from the General Biological Supply House, Inc.

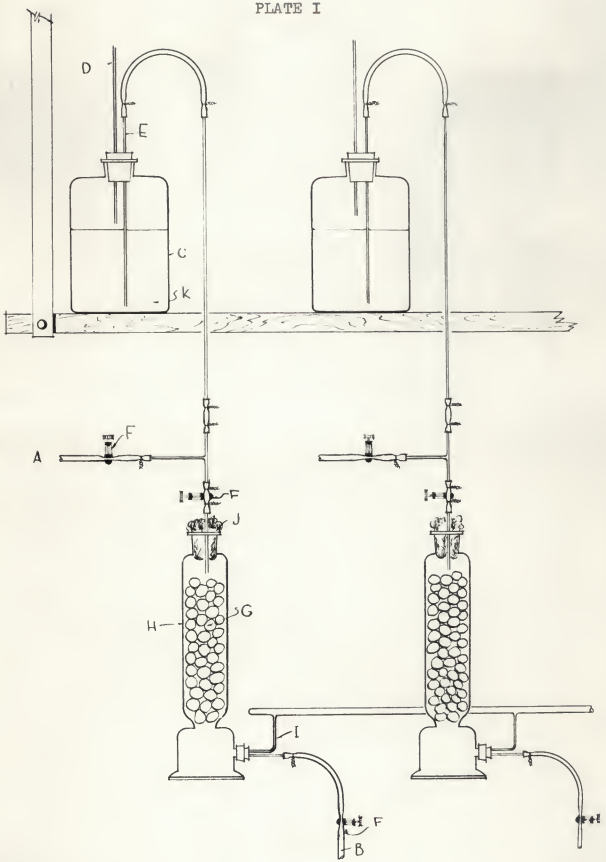
Slime Material

Zoogleal films were scraped off stones of trickling filter ballast with a knife blade and placed on a microscopic slide. Observations were made of the protozoan population and other metazoans which were present in the films by means of a wide-field binocular and regular microscope. A drop of methyl cellulose was added to each film to slow down the movement of the organisms so they could be easily observed.

EXPLANATION OF PLATE I

A. Sampling point for unfiltered sample.
B. Sampling point for filtered sample.
C. Reservoir jug.
D. Air vent.
E. Siphon.
F. Screw clamp.
G. Crushed ballast stones with biologic film.
H. Calcium chloride drying tower.
I. Compressed air line.
J. Cotton plug.
K. Sewage-culture mixture.

PLATE I



Protozoan Cultures

Trickling filter stones were placed in a pint-size jar. Tap water was added to the jar. The jar was shaken to dislodge the organisms from the stones. A drop of the mixture was placed on a microscopic slide, and pure cultures of Paramecium spp. were isolated with the aid of a wide-field binocular microscope and capillary pipettes. The isolated Paramecium spp. cells were placed in 1000 ml. Erlenmeyer flasks containing about 800 ml. tap water. Three pellets of commercial rabbit feed were added to each of the flasks. The inoculated flasks were incubated at room temperature until sufficient growth was obtained.

Bacterial Cultures

The test cultures consisted of two coliform species (Escherichia coli, Aerobacter aerogenes) and three Salmonella organisms (S. paratyphi, S. schottmuelleri, and S. typhosa). These organisms were obtained from the stock culture collection of the Department of Bacteriology of Kansas State College.

Nutrient agar slants were inoculated with the test organisms. These were incubated at room temperature for 24 hours then cooled to approximately 12° C. A five ml. portion of sterile, distilled water which had been previously cooled to 12° C. was added to each slant. The organisms growing on the surface of the nutrient agar slants were scraped off and the mixture agitated vigorously with the inoculating needle to obtain a smooth suspen-

sion. These suspensions were used as the prepared inocula in Experiment I. Dilutions of 1-100 and 1-10,000 were made for each of the test organisms from the suspension previously prepared. The 1-10,000 dilutions were used as the inocula for Experiments II, III, IV, V, VI, VII, and VIII.

EXPERIMENT I

The Destruction of Coliform Organisms and Salmonella Species in Passing Through a Trickling Filter

It had been shown in the past by other investigators that trickling filters were effective in removing a large percentage of the bacterial cells present in sewage. However, most of the work dealt with coliform organisms as a whole and with S. typhosa and not with other members of the genus Salmonella.

Procedure

Samples of ballast stones were taken from the filter bed. These were broken into smaller sizes (one-half to one inch in diameter) by a stone-crushing machine, placed in a perforated bucket, and allowed to stand in the trickling filter for one week. The crushed stones were then placed into the experimental trickling filter.

Samples of sewage were taken from the rotating arms of the

Fort Riley trickling filter and placed in five-gallon cans.[1] Care had to be taken to keep the temperature of both the sewage and stones at approximately 12° C. -- this being the average temperature of the trickling filter effluent of the Fort Riley sewage treatment plant. The collected sewage was poured into five one-gallon jugs. Each of the jugs was inoculated with one ml. of a different prepared culture inoculum. The contents of the inoculated jugs were then swirled vigorously by hand to distribute the inoculum evenly. The jugs were then set on a suspended shelf above the experimental trickling filters. The sewage was allowed to flow at a slow rate to permit the protozoans present on the filter stones to destroy the bacteria present in the sewage (Plate I). Air was forced through the bottom of the filter to maintain aerobic conditions.

One hundred ml. of sample was collected at the end of 30 minutes at outlet A (Plate I). This was labelled as sample A. Immediately after collecting sample A, another 100 ml. portion was collected at outlet B. This was labelled sample B.

At the end of a two-hour period, the entire sampling procedure was repeated. The samples obtained were labelled A_1 and B_1.

Eosin Methylene Blue (E.M.B.) agar was used as the medium for enumerating the coliform organisms. It was selected because E. coli and A. aerogenes can be easily distinguished from each other and from non-coliform organisms. Bismuth sulfite agar was

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[1] The sample taken from the rotating arms is essentially the effluent from the primary sedimentation tank.

selected for enumerating the Salmonella species because it is a highly selective medium for Salmonella organisms. S. typhosa and S. schottmuelleri produce black colonies, while S. paratyphi yields slightly raised green colonies.

From samples A and A_1 equivalents of 0.001, 0.0001, 0.00001, and 0.000001 ml. amounts were planted. E.M.B. agar, liquefied and cooled to 48° C., was poured into the plates containing the samples of coliform organisms, and bismuth sulfite agar was similarly poured into plates containing the Salmonella organisms. The plates were incubated at 37° C. for 48 hours. The colonies on the plates were counted by using a Quebec colony counter and a Veeder hand tally.

The procedure was repeated with samples B and B_1, but equivalents of 0.01, 0.001, 0.0001, and 0.00001 ml. amounts were used.

Results

The results are presented in Table 1. The values are the averages of three trials. Destruction of the organisms varied from 57 to 90 per cent. The Salmonella species proved to be the more susceptible organisms. The works of Jordan et al (1904) and Russell and Fuller (1906) showed that E. coli is an organism which can resist the adverse conditions of water and sewage better than S. typhosa. A. aerogenes, like E. coli, is also a hardy organism in sewage and was consequently found to be more resistant. The Salmonella organisms tested showed a high rate of destruction.

Table 1. The per cent reduction of coliform organisms and Salmonella species suspended in fresh settled sewage when passed through experimental trickling filters. (Averages of three trials.)

Organisms	Per cent reduction after 30 min.	Per cent reduction after 2 hours
A. aerogenes	57.5	63.2
E. coli	64.4	73.7
S. paratyphi	88.9	81.05
S. schottmuelleri	90.8	82.5
S. typhosa	78.5	83.4

EXPERIMENT II

The Effect of Unsterilized Sewage on Coliform Organisms and Salmonella Species

In Experiment I, it was shown that both the coliform organisms and Salmonella species were being destroyed in passing through trickling filters. However, destruction occurred at different rates. The next step was to determine the varying viability of the test organisms in unsterilized sewage.

Procedure

Five six-ounce bottles were filled with previously cooled (12° C.), unsterilized sewage. Each bottle was inoculated with one ml. of a different culture inoculum, and was then thoroughly

shaken by hand to obtain a uniform suspension. One ml. and 0.1 ml. samples were taken immediately. The bottles were held at 12° C. and similar samples were taken at the end of 30 minutes, 1, 2, 8, 24, 48, 72, 96, and 120 hours.

E.M.B. agar, liquefied and cooled to 48° C., was used for the coliform organisms, and bismuth sulfite agar similarly prepared was used for the Salmonella species. The inoculated plates were incubated at 37° C. for 48 hours. The characteristic colonies on the plates were enumerated by means of a Quebec colony counter and Veeder hand tally.

Results

The results of this experiment are plotted on the graph in Fig. 1. The plotting is based on the averages of three trials.

The survival of coliform organisms and Salmonella species varied greatly. The number of viable cells per ml. of A. aerogenes and E. coli decreased slowly during the five days the organisms were being tested. On the contrary, the number of viable cells of Salmonella species tested diminished more rapidly for the first 24 hours after which the population became stabilized. S. paratyphi was destroyed at a faster rate than S. schottmuelleri and S. typhosa.

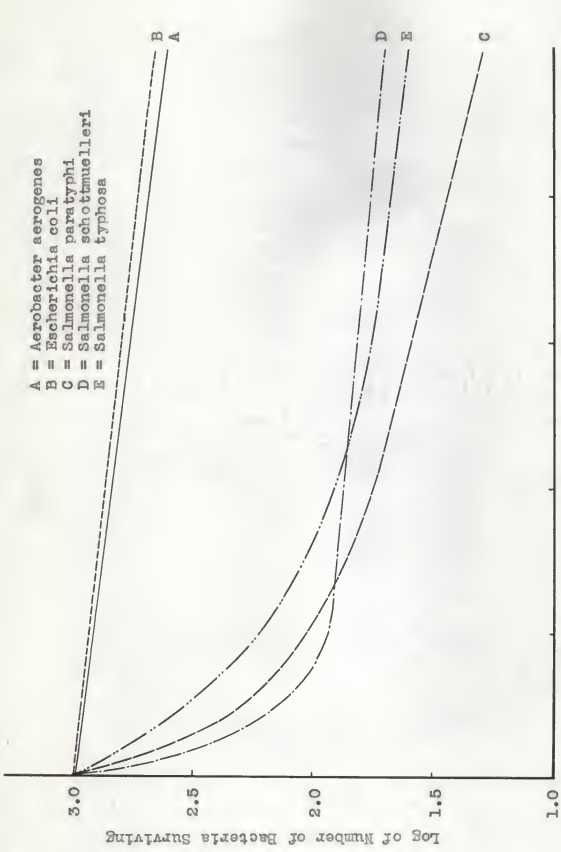

Fig. 1. Graph showing the rate of destruction of test organisms in unsterilized sewage.

EXPERIMENT III

The Effect of Sterile, Distilled Water on Coliform Organisms and *Salmonella* Species

It was demonstrated in both Experiments I and II that the coliform organisms tested were more resistant than *Salmonella* species to the adverse conditions of sewage and biologic antagonism of the slime on stones of trickling filter ballast. Other investigators in the past have demonstrated that *S. typhosa* was more susceptible to destruction in sterile water than *E. coli*. However, no report could be found pertaining to similar work having been done with other organisms in the genus *Salmonella*.

Procedure

The procedure in this experiment was the same as that of Experiment II except that the five six-ounce bottles were filled with sterile, distilled water instead of unsterilized sewage.

Results

The results of the experiment are presented on the graph in Fig. 2. The graph is based on the averages of three trials.

Again the general trend showed *A. aerogenes* and *E. coli* to be very resistant organisms in contrast to the apparently more susceptible members of the *Salmonella* species when stored in sterile, distilled water. The *Salmonella* organisms tested were less

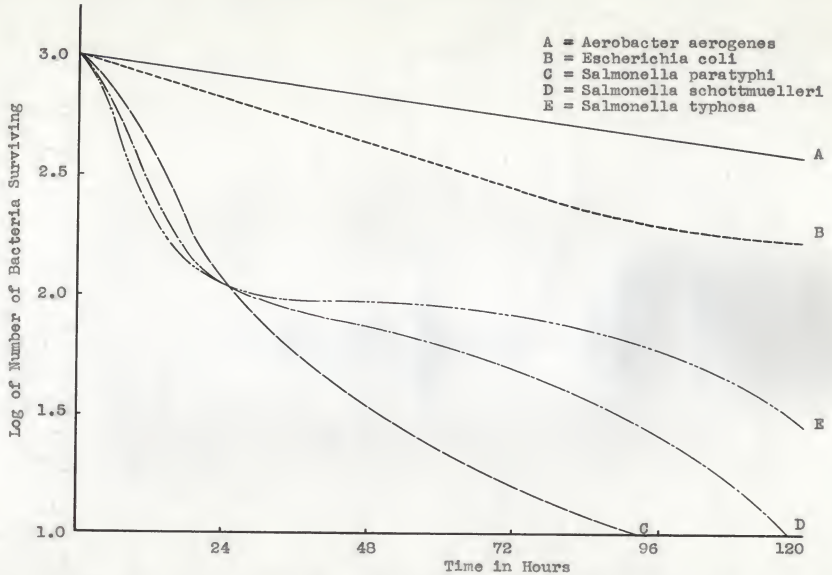

Fig. 2. Graph showing the rate of destruction of test organisms in sterile, distilled water.

viable than the more resistant coliform organisms. As in Experiment II, S. paratyphi here also appeared to have less resistance to the conditions in sterile, distilled water than either S. schottmuelleri and S. typhosa.

EXPERIMENT IV

The Effect of Filtered Sewage on Coliform Organisms and Salmonella Species

It was found in Experiments I and II that the test organisms were being reduced in trickling filters and raw sewage. At this point it was necessary to determine if the action of specific bacteriophages was responsible for the reduction of the test organisms in previous tests.

Procedure

The procedure in this experiment was the same as that of Experiment II except that bacteria-free filtrates of sewage were used instead of fresh, raw sewage. The filtrates were obtained by filtering the sewage through a series of filters: first through gauze, then through coarse filter paper, and finally through a sterile Berkefeld filter.

Results

The results of the experiment are shown in Fig. 3. The data presented in the graph are based on the averages of two trials.

Bacteriophage specific for S. paratyphi may have been present in the filtered sewage. A noticeable drop in numbers of S. paratyphi occurred in 48 hours. On the other hand, this decrease in numbers may have been due to the presence of chemicals toxic to this particular organism.

It is evident from the results that specific bacteriophages for the rest of the test organisms were absent from or were in insignificant amounts in the sewage. The filtrates probably contained many beneficial nutrients and were apparently free from antagonistic agents. Consequently, the filtered sewage actually supported good growth of the coliform organisms. However, all of the test organisms showed a definite drop in numbers before exhibiting increase in growth. A longer lag phase occurred in the case of the Salmonella species, which may be due to the need for a longer period of adjustment by the organisms to the new environment.

EXPERIMENT V

The Effect of Heated, Filtered Sewage on Coliform Organisms and Salmonella Species

After finding in Experiment IV that a noticeable drop in numbers of S. paratyphi occurred in 48 hours in filtered sewage, it

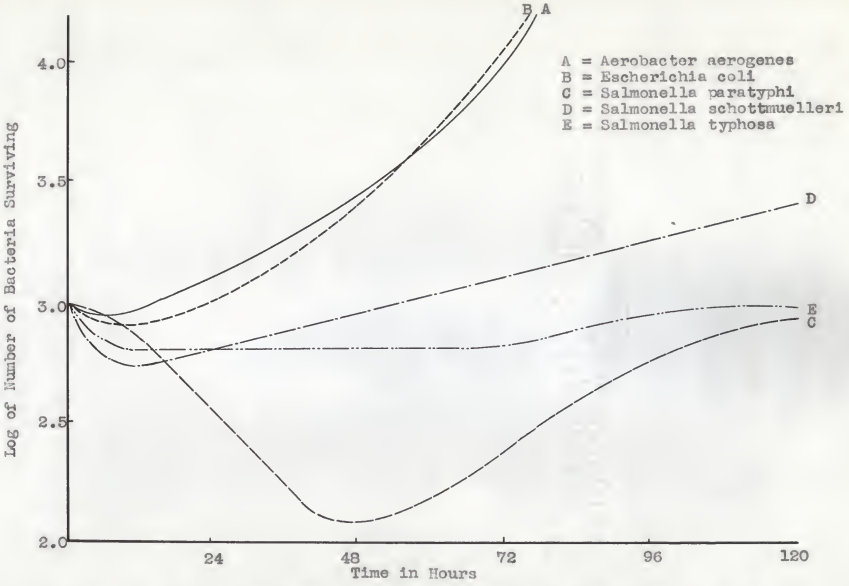

Fig. 3. Graph showing the rate of destruction of test organisms in filtered sewage.

was necessary to determine the basic reason for this drop. The decrease may be due to the presence of bacteriophage specific for S. paratyphi or to the presence of chemicals in the filtered sewage.

Procedure

The procedure in this experiment was the same as that of Experiment IV except that heated, bacteria-free filtrates of sewage were used instead of fresh, raw sewage. The heated filtrates were obtained by filtering the sewage through the same series of filters as in the procedure in Experiment IV. The filtrates were then heated to boiling, boiled for one minute, and finally cooled in a cold water bath.

Results

The results of the experiment are shown in Fig. 4. The data presented in the graph are based on the averages of two trials.

Toxic chemicals were probably present in insignificant quantities or were totally absent from the sewage. The heated, filtered sewage provided the coliform organisms with a medium which was suitable for growth. By 72 hours the plates containing the coliform organisms were uncountable. S. paratyphi phage, if present in the sewage, was destroyed by heating. Consequently, only a small decrease in number of S. paratyphi occurred. Little growth was obtained with the Salmonella organisms. All of the test organisms exhibited a definite drop in numbers after which

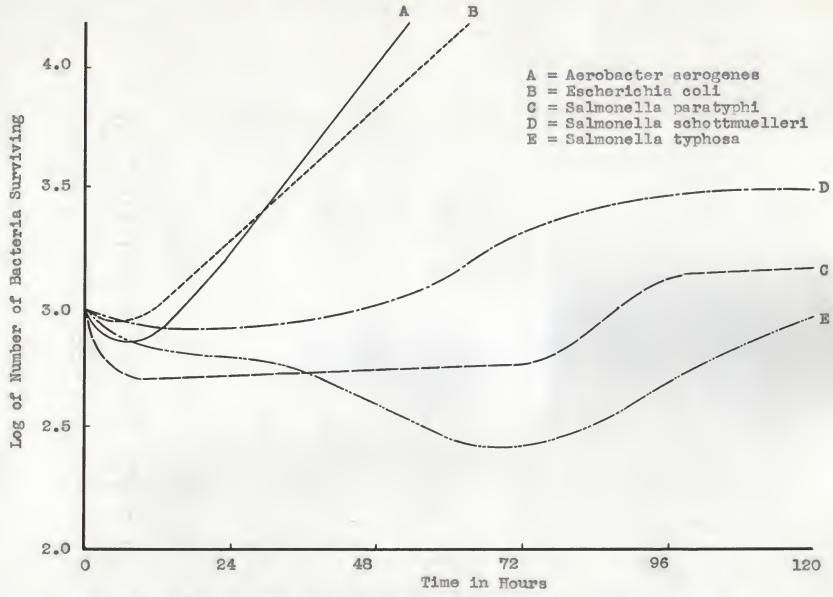

Fig. 4. Graph showing the rate of destruction of test organisms in heated, filtered sewage.

a small increase in growth occurred.

EXPERIMENT VI

The Effect of Agitation and of the Biologic Film of Trickling Filter Ballast upon Coliform Organisms and Salmonella Species Suspended in Sterile, Distilled Water

In Experiment I, it was shown that trickling filters were effective in removing large percentages of the coliform organisms and Salmonella species from sewage as it passed over the filter ballast. The object of this experiment was to provide further proof that the microorganisms comprising the film on the stones of the trickling filter ballast were responsible for the high rate of destruction of the test organisms.

Procedure

Stone samples were taken from the trickling filter bed of the Fort Riley sewage treatment plant, broken into smaller sizes (one-half to one inch in diameter), and replaced to accumulate a new slime coating. When a slime coating had been established, five pieces of crushed stones were placed into five 300 ml. Erlenmeyer flasks containing 100 ml. sterile, distilled water. Each of the five flasks was inoculated with one ml. of a different culture inoculum. Samples of 1.0 ml. and 0.1 ml. amounts were taken immediately. The inoculated flasks were then placed on a Kline shaker. The speed of the shaker was set at 110 rota-

tions per minute. Similar samples were taken at time intervals of 1, 2, 3, 4, 5, and 6 hours.

E.M.B. agar which had been liquefied and cooled to 48° C. was used for A. aerogenes and E. coli and bismuth sulfite agar similarly prepared was used for the Salmonella organisms. The inoculated plates were incubated at 37° C. for 48 hours. The colonies which were characteristic of the test organisms on the plates were enumerated by using a Quebec colony counter and a Veeder hand tally.

Results

The rates of destruction of all the organisms tested are shown on the graph in Fig. 5. The coliform organisms were destroyed at a slow rate, while the more susceptible Salmonella species were destroyed at a considerably faster rate. The overall results show that the biologic film on the stones of trickling filter ballast is capable of removing the test organisms from sterile, distilled water; especially is this true of members of the Salmonella species.

EXPERIMENT VII

The Effect of Paramecium spp. in the Biologic Film of Trickling Filter Ballast upon Coliform Organisms and Salmonella Species Suspended in Sterile, Distilled Water

It was shown in Experiments I and VI that the biologic film on the stones of trickling filter ballast was capable of removing

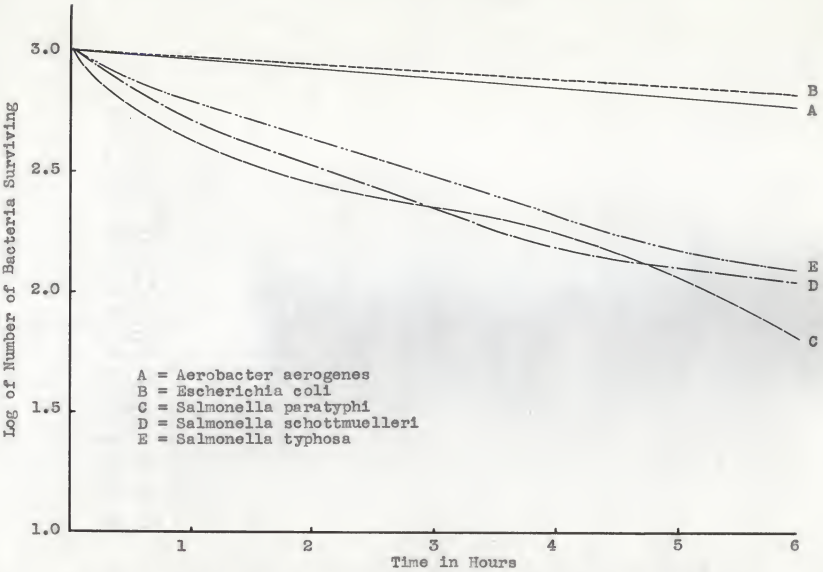

Fig. 5. Graph showing the rate of destruction of test organisms by micro-organisms in the biologic film in the stones of trickling filter ballast in sterile, distilled water.

bacterial organisms from sterile, distilled water and sewage. In this experiment an effort was made to measure the effect of the biologic film, containing a known number of Paramecium cells per ml., upon the viability of test organisms.

Procedure

Stone samples were taken from the trickling filter bed of the Fort Riley sewage treatment plant and placed in a jar. The biologic film on the stones was scraped off with a knife and placed into another jar containing 10 ml. sterile, distilled water and shaken vigorously. A drop of the mixture was transferred to a hemacytometer by using a serological pipette, and the number of Paramecium spp. in the film was enumerated.

Five six-ounce bottles containing 100 ml. sterile, distilled water were inoculated with one ml. of suspension of a different culture inoculum prepared as described under Experimental Materials. One ml. of the biologic film (Paramecium spp. enumerated) was added to each bottle. Samples of 0.01, 0.001, and 0.0001 ml. amounts were taken immediately for the coliform organisms, and samples of 0.1, 0.01, 0.001 ml. amounts were taken for the Salmonella species. Similar samples were taken at time intervals of 1, 2, 3, 4, 5, and 6 hours.

E.M.B. agar which had been liquefied and cooled to 48° C. was used for the coliform organisms, and bismuth sulfite agar similarly prepared was used for the Salmonella species. The inoculated plates were incubated at 37° C. for 48 hours. The charac-

teristic colonies on the plates were enumerated the same way as in Experiment VI.

Results

The number of Paramecium spp. in the biologic film was found to be 10,000 per ml.

Again, as in previous experiments, the coliform organisms were found to be more resistant than members of the Salmonella species. The rate of destruction is illustrated in Fig. 6. The differences in the rates of destruction were not as great in this experiment as in previous experiments.

EXPERIMENT VIII

The Effect of a Purified Suspension of Paramecium spp. upon Coliform Organisms and Salmonella Species

In Experiment VII, the effect of the total population of organisms in the biologic slime on the stones of trickling filter ballast was determined with the number of Paramecium spp. in the slime being recorded. In this experiment, the same number of Paramecium spp. was used without the presence of the other protozoans and metazoans. By following the same procedure it could be determined whether the Paramecium spp. was one of the protozoans in the biologic slime which was primarily responsible for the destruction of the test organisms in sterile, distilled water and sewage.

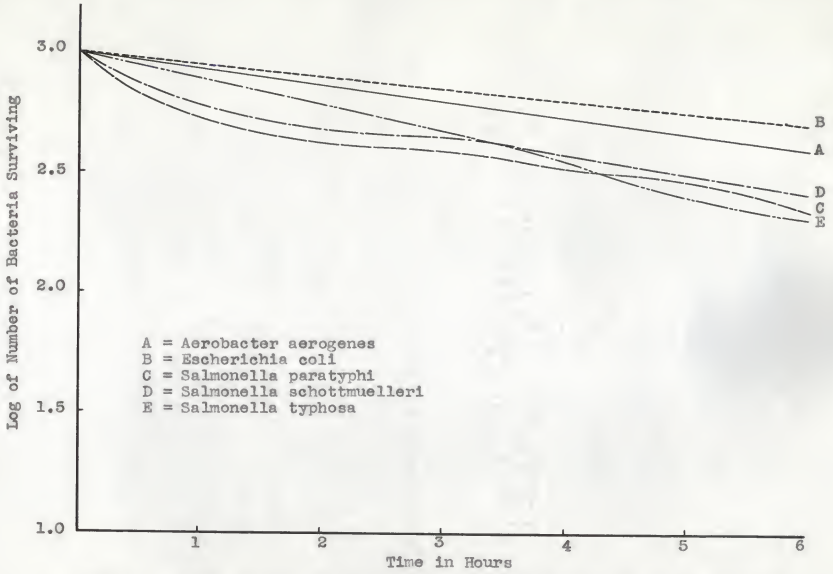

Fig. 6. Graph showing the rate of destruction of test organisms by total microbial population containing a known quantity of Paramecium spp. in sterile, distilled water.

Procedure

A pure culture of *Paramecium spp.* which had been isolated from the biologic slime on the stones of trickling filter ballast was concentrated to 10,000 cells per ml. by filtering the culture through coarse filter paper and by enumerating the *Paramecium spp.* by use of the hemacytometer.

One ml. of a different culture inoculum was added to five six-ounce bottles containing 100 ml. sterile, distilled water. Ten thousand cells of *Paramecium spp.* was added to each bottle. Samples of 0.01, 0.001, and 0.0001 ml. amounts were taken immediately for *A. aerogenes* and *E. coli*, while samples of 0.1, 0.01, and 0.001 ml. amounts were taken for the *Salmonella* organisms. Similar samples were taken at time intervals of 1, 2, 3, 4, 5, and 6 hours.

E.M.B. agar, liquefied and cooled to 48° C., was used for culturing the coliform organisms, and bismuth sulfite agar similarly prepared was used for the *Salmonella* species. The colonies which were characteristic of the test organisms were enumerated by using a Quebec colony counter and Veeder hand tally.

Results

The results of this experiment are shown in Fig. 7. All of the test organisms were slowly destroyed by the *Paramecium spp.* but at different rates. As in previous experiments, the *Salmonella* species were destroyed at higher rates than the more resistant coliform organisms.

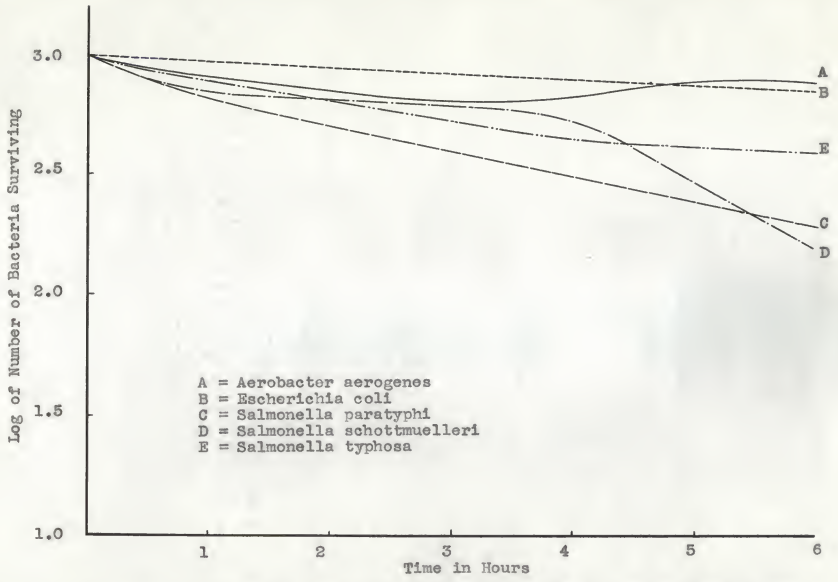

Fig. 7. Graph showing the rate of destruction of test organisms by purified suspension of Paramecium spp. in sterile, distilled water.

DISCUSSION

The main objective of this research was to attempt to determine if the protozoans present in the biologic slime on the stones of trickling filter ballast would destroy Salmonella typhosa, Salmonella paratyphi, and Salmonella schottmuelleri.

Before the problem was started, an experimental trickling filter was set up and tests were made of the efficiency of the removal of enteric bacteria. The underlying principle in the trickling filter is to provide a site where a variety of microorganisms and oxygen can come in close contact with sewage components, including sewage bacteria. The biologic slime is made up of many species of bacteria, protozoans, insect larvae, and free-living roundworms. The more predominant protozoans present were found to be species of Vorticella, Paramecium, Chilodon, Opercularia, Oxytricha, and Amoeba.

It was found that the experimental filter was very efficient in removing the test organisms present in sewage. Comparative tests showed that coliform organisms were destroyed at a much slower rate than the Salmonella organisms. The test organisms were then studied to determine the rate of destruction in unsterilized sewage and sterile, distilled water. These tests again showed that Salmonella species were more susceptible to destruction in both environments than the coliform organisms.

The question then arose, "Might the possibility of bacteriophages specific for the test organisms account for the dying out of the bacteria?" Bacteria-free filtrates of sewage were tested

against enteric bacterial organisms. The results of the tests showed that a considerable decrease in cells occurred with S. paratyphi. This decrease may have been due to the presence of bacteriophages, or to the presence of chemicals toxic to this particular organism. Consequently, another test was made. Bacteria-free filtrates were heated to destroy any bacteriophages which might have been present in the sewage. The heated, filtered sewage provided the coliform organisms with a medium suitable for good growth. After exhibiting an initial decrease in numbers, the Salmonella species began growing in the heated, filtered sewage. Similar results were obtained with filtered sewage. The number of S. paratyphi cells did not decrease as much as with the unheated filtered sewage, which suggests the possibility of S. paratyphi phage being present in the sewage.

In further efforts to verify the destructive properties of the biologic film on coliform organisms and members of the Salmonella species, stones of trickling filter ballast coated with the slime were tested. Destruction of the test organisms occurred in varying degrees. The coliform organisms were destroyed at a slow rate, and the more susceptible Salmonella species were destroyed at a much faster rate. Then the biologic film was scraped off the stones and the number of cells of Paramecium spp. present was enumerated. The scrapings were added to suspensions of the test bacterial organisms. Again the coliform bacteria proved to be the more resistant organisms. However, higher rates of destruction occurred with the tests of the stones of trickling filter ballast and the bacterial organisms than with the scrap-

ings of biologic film on the stones of trickling filter ballast. An explanation of these findings may be that, in the latter test, the Erlenmeyer flasks were not placed on a Kline shaker. Consequently, the sessile protozoans (Vorticella and Opercularia sp.) were not as effective in destroying bacteria as when the flasks were agitated slowly. Possibly only the motile protozoans were fully engaged in destroying the test organisms.

Another experiment was run with a pure culture of Paramecium spp. using the same number of cells as in the previous experiment. By running this test it could be determined whether Paramecium spp. was primarily responsible for the destruction of the test organisms or whether other organisms in the slime were accounting for the major decrease of the test bacteria. The results showed that about half of the reduction could be attributed to Paramecium spp. as is shown in Figs. 8 through 12.

In every experiment A. aerogenes and E. coli proved to be more resistant than the apparently more susceptible members of the Salmonella species. The application of this information is important in the standard examination of water and sewage. A. aerogenes, when present in water, indicates soil contamination. The presence of E. coli in water and sewage indicates fecal contamination with the possibility of enteric pathogens being present. When a sample of water examined contains no E. coli, it is very probable that members of the Salmonella species would be absent. Another application of the fact that coliform bacteria are more resistant to destruction than the more susceptible Salmonella organisms is in the treatment of sewage. Approximately

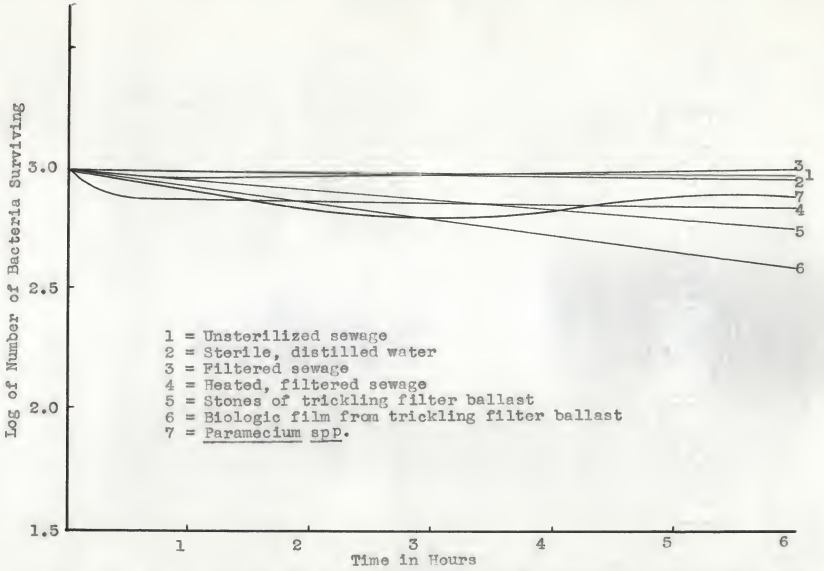

Fig. 8. Graph showing the rate of destruction of Aerobacter aerogenes in the presence of: unsterilized sewage; sterile, distilled water; filtered sewage; heated, filtered sewage; stones of trickling filter ballast; biologic slime from trickling filter ballast; and Paramecium spp.

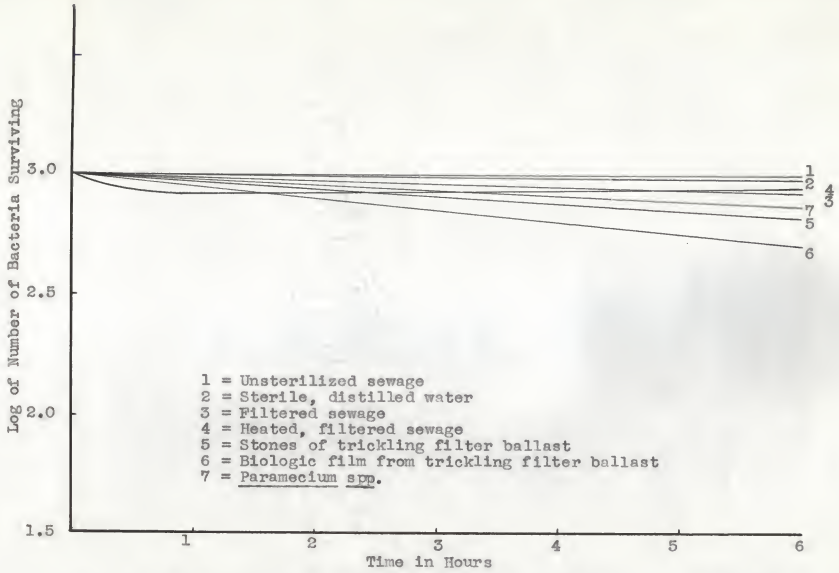

Fig. 9. Graph showing the rate of destruction of Escherichia coli in the presence of: unsterilized sewage; sterile, distilled water; filtered sewage; heated, filtered sewage; stones of trickling filter ballast; biologic slime from trickling filter ballast; and Paramecium spp.

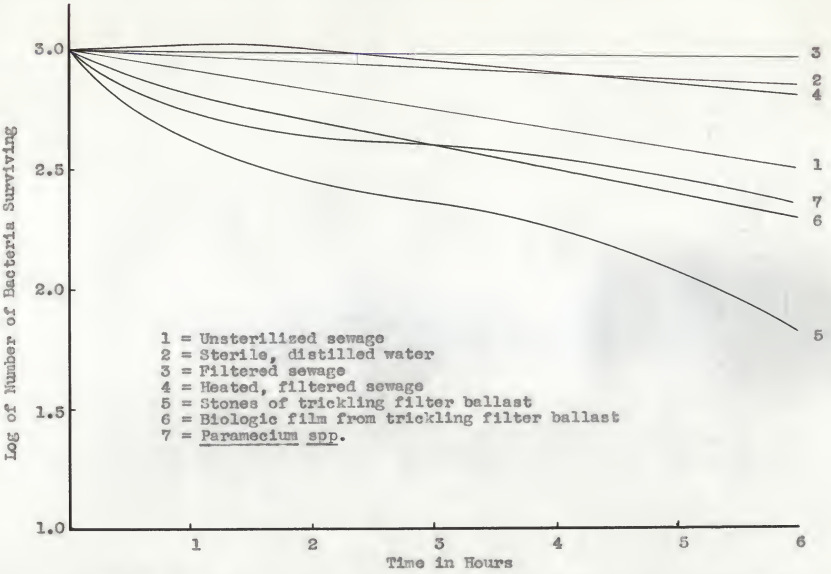

Fig. 10. Graph showing the rate of destruction of Salmonella paratyphi in the presence of: unsterilized sewage; sterile, distilled water; filtered sewage; heated, filtered sewage; stones of trickling filter ballast; biologic slime from trickling filter ballast; and Paramecium spp.

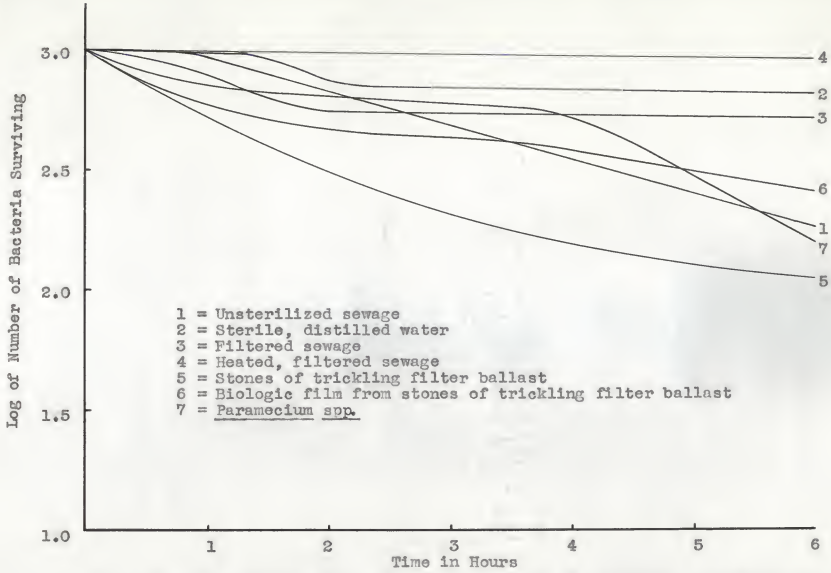

Fig. 11. Graph showing the rate of destruction of Salmonella schottmuelleri in the presence of: unsterilized sewage; sterile, distilled water; filtered sewage; heated, filtered sewage; stones of trickling filter ballast; biologic slime from stones of trickling ballast; and Paramecium spp.

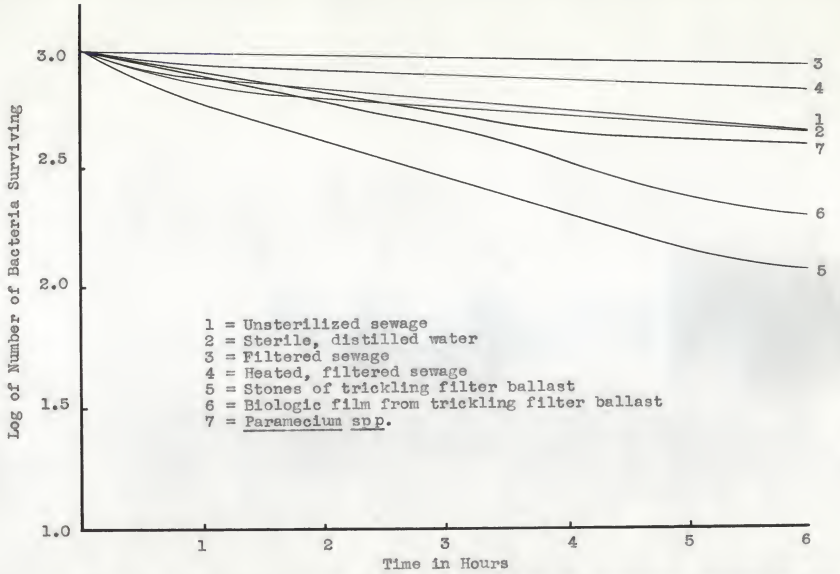

Fig. 12. Graph showing the rate of destruction of Salmonella typhosa in the presence of: unsterilized sewage; sterile, distilled water; filtered sewage; heated, filtered sewage; stones of trickling filter ballast; biologic slime from trickling ballast; and Paramecium spp.

50 to 70 per cent of the coliform organisms were removed, and approximately 80 to 90 per cent of the Salmonella species were destroyed in the experimental trickling filter. These figures are the results of inoculating large numbers of test bacterial organisms into the influents of the experimental filter. In a trickling filter like the one at the Fort Riley sewage treatment plant it can be said that, at most, only very low number of pathogens will pass through the filter bed without being destroyed by the microorganisms in the biologic film on the stones of the trickling filter ballast.

In an attempt to run a food preference test for protozoan organisms on a nutrient agar plate, it was found that many free-living roundworms were present in the Petri dish. It is suspected by the author that these worms may also have a significant effect on bacteria. Time has not permitted the testing of the effect of roundworms present in the biologic film on bacteria. It is suggested that a pure culture of the free-living roundworms be obtained from the biologic slime on the stones of the trickling filter ballast. Eggs can be obtained from the roundworms. The adult worms, together with other contaminating microorganisms, can then be killed with some quaternary ammonium compound or antibiotics. The eggs can be washed with sterile, distilled water, enumerated by means of a hemacytometer, and tested against bacterial organisms.

SUMMARY

1. A study has been made on the effects of protozoan fauna of trickling filter ballast on the following organisms: Aerobacter aerogenes, Escherichia coli, Salmonella paratyphi, Salmonella schottmuelleri, and Salmonella typhosa.

2. The more predominant protozoans present in the biologic slime on the stones of trickling filter ballast were found to be species of Vorticella, Paramecium, Chilodon, Opercularia, Oxytricha, and Amoeba. Many species of bacteria, insect larvae, and free-living roundworms were also present.

3. The trickling filter is efficient in removing bacteria from sewage. Approximately 55 to 70 per cent of the coliform bacteria and about 80 to 90 per cent of the Salmonella organisms were destroyed in passing through a trickling filter.

4. The biologic film on the stones of trickling filter ballast reduced the number of the test organisms. The coliform bacteria were destroyed at a slow rate, while the more susceptible members of the Salmonella species were destroyed at a much faster rate.

5. Of the total microbial population in the biologic slime on the stones of trickling filter ballast, Paramecium spp. is responsible for a large part of the decrease in the number of bacteria. This overall decrease was, however, small under the test conditions.

6. A. aerogenes and E. coli were more resistant to destruction than S. paratyphi, S. schottmuelleri, and S. typhosa in un-

sterilized sewage and sterile, distilled water.

7. Bacteriophage for S. paratyphi was probably present in the sewage. In the absence of phage, the filtered sewage served as a medium for the growth of the test organisms.

8. Chemicals toxic to Salmonella species were absent or were in insignificant amounts in sewage. Heated, filtered sewage enhanced the growth of coliform organisms. Salmonella species exhibited an initial decrease in numbers before growing.

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THE EFFECTS OF THE PROTOZOAN FAUNA OF TRICKLING FILTER BALLAST UPON SALMONELLA SPECIES

by

WILFRED V. G. CHONG

B. S., Gustavus Adolphus College, 1951

AN ABSTRACT OF A THESIS

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Department of Bacteriology

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OF AGRICULTURE AND APPLIED SCIENCE

1953

Trickling filters are often employed as part of the secondary treatment of sewage. They function in the removal of unstable chemical materials and in the reduction of the bacterial population in sewage. The basic principle of the trickling filter is the providing of a site where a variety of aerobic microorganisms and oxygen can come in close contact with the sewage components, including sewage bacteria.

Early workers at the Lawrence (Massachusetts) Experiment Station (1885 to 1900) had found that intermittant sand filters were effective in removing 98 to 99 per cent of the organic matter and bacteria in sewage. It was also found at the Lawrence Experiment Station that Bacterium coli (Escherichia coli) was more resistant to the adverse condition of sand filters than Eberthella typhi (Salmonella typhosa). Many workers had showed an ecological relationship between the bacterial and protozoan populations. It was noted by these investigators that protozoa did have detrimental effects on bacteria.

The primary objective of this research was to attempt to determine whether the protozoan population in the biologic slime on the stones of trickling filter ballast was responsible for the destruction of enteric pathogenic bacteria in sewage.

Experimental trickling filters were set up in the laboratory and tests were made of their efficiency in the removal of selected enteric bacteria. The organisms tested were Salmonella paratyphi, Salmonella schottmuelleri, and Salmonella typhosa. Escherichia coli and Aerobacter aerogenes were also tested as control organisms for comparative purposes. The trickling filters

proved to be very efficient in removing the test organisms present in sewage. Approximately 55 to 70 per cent of the coliform organisms and about 80 to 90 per cent of the Salmonella species tested were destroyed in passing through the trickling filters.

Studies were then made of the test organisms to determine the rate of their destruction in unsterilized sewage and sterile, distilled water. Again, the tests demonstrated that Salmonella species were more susceptible to destruction in both environments than the coliform organisms.

Bacteria-free filtrates of sewage were tested against the enteric bacteria to explore the possibility of specific bacteriophages being responsible for the destruction of bacteria. A considerable decrease in cells occurred with S. paratyphi. However, this decrease may have been due to the presence of toxic chemicals to this particular organism in the sewage. Consequently, heated bacteria-free filtrates which contained no bacteriophage were tested against the test organisms. The heated, filtered sewage provided the coliform organisms with a medium suitable for good growth. The Salmonella species showed an initial decrease in numbers before exhibiting growth in the heated, filtered sewage. The decrease in the number of S. paratyphi cells was insignificant in contrast to the large reduction in unheated filtered sewage, which suggested the possibility of the presence of S. paratyphi phage in the sewage.

Stones of trickling filter ballast coated with slime were tested to determine the destructive properties of the biologic film on coliform organisms and the test Salmonella species. The

flasks containing the stones and bacteria were then placed on a Kline shaker. The results showed that coliform organisms were destroyed at a slow rate, and Salmonella species were destroyed at a much faster rate. Then biologic film was scraped off the stones and the number of cells of Paramecium spp. present was determined. The scrapings were added to suspensions of the test bacterial organisms. Again the Salmonella species appeared as the more susceptible organisms. Higher rates of destruction occurred in the experiments using the trickling filter stones and the test bacterial organisms than in the experiments using scrapings of the biologic film from the stones of trickling filter ballast. This may have been due to the agitation of the trickling filter stones and the test organisms which permitted more bacteria to come in contact with sessile forms of protozoans.

In the final experiment a pure culture of Paramecium spp. using the same number of cells as in the previous experiment was added to suspensions of the test bacterial organisms. The reason for the test was to determine whether Paramecium spp. was primarily responsible for the destruction of the test organisms or whether other organisms in the slime were accounting for the major decrease of the test bacteria. The results showed that about half of the reduction could be attributed to Paramecium spp. However, under the test conditions the overall reduction was small.

SUMMARY

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